## REMARKS

**1. Support for amendments and new claims**

The claim amendments are supported throughout the application as filed. Amendments to claim are supported, for example, in the claim as originally filed. The phrase "easily or inherently form aggregates during culturing" is supported, for example, on page 3 line 35 to page 4 line 1 of the application as filed. Recitation of "the pores" of the filter module is supported, for example, on page 2 lines 6-11 culturing" is supported, for example, on page 3 line 35 to page 4 line 1 of. The amendment to claim 4 is supported, for example on page 5 lines 14-17 culturing" is supported, for example, on page 3 line 35 to page 4 line 1. The amendment to claim 15 is supported, for example in the paragraph bridging page 6-7 of the application as filed, in light of the teachings of the specification as a whole. Thus, the amendments to the claims do not introduce any new matter.

**2. Claim rejections under 35 USC §101**

The Patent Office rejected claims 1-9 and 11-15 under 35 USC §101 as being drawn to non-statutory subject matter. Specifically, the Patent Office asserted that the claims can be interpreted to not require any active method steps. Applicants traverse this rejection, but have nonetheless amended the claims to clearly recite active method steps. Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

**3. Claim rejections under 35 USC §112**

**(a)** The Patent Office rejected claim 1 under 35 USC §112, second paragraph, as being indefinite, based on the assertion that it does not clearly recite any method steps. Applicants traverse, but have nonetheless amended the claim to clearly recite active method steps. Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

**(b)** The Patent Office rejected claim 1 under 35 USC §112, second paragraph, as being indefinite, based on the assertion that recitation of "reducing cell aggregation" is unclear. Applicants traverse, but have nonetheless amended the claim to recite "limiting", thus obviating the issue of whether there is an adequate point of

reference for recitation of "reducing." Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

**(c)** The Patent Office rejected claim 1 under 35 USC §112, second paragraph, as being indefinite, based on the assertion that recitation of "wherein no more than 5% of the animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing" is confusing because it is not clear if this requires some additional step. Applicants traverse this rejection. The amended claims clearly recite that the recited method steps result in limiting cell aggregation, such that no more than 5% of the animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing. Thus, the claim would be clear to those of skill in the art, and Applicants respectfully request reconsideration and withdrawal of the rejection.

**(d)** The Patent Office rejected claim 1 under 35 USC §112, second paragraph, as being indefinite, based on the assertion that recitation of "resulting in an outflow of liquid having a lower animal cell density than the cell culture" is confusing because it is not clear if this requires some additional step. Applicants traverse this rejection. The amended claims clearly recite that the recited continuous perfusion culturing steps result in an outflow of liquid through the pores of the filter module having a lower animal cell density than the cell culture prior to circulating through the filter module. Thus, the claim would be clear to those of skill in the art, and Applicants respectfully request reconsideration and withdrawal of the rejection.

**(e)** The Patent Office rejected claim 1 under 35 USC §112, second paragraph, as being indefinite, based on the assertion that recitation of "an outflow" is not clear, as the claim does not recite any particular apparatus. As would be readily apparent to those of skill in the art, an "outflow" of liquid as recited in the claim refers to liquid that has passed through the pores of the filter. Applicants have amended the claims to make this even clearer. Thus, the claim would be clear to those of skill in the art, and Applicants respectfully request reconsideration and withdrawal of the rejection.

**(f)** The Patent Office further commented on how claim 1 might be interpreted, and asserted that even under such an interpretation, the claim was indefinite in terms of what method steps were required. The Patent Office further required clarification by claim amendment. Applicants traverse the rejection, but solely in order to expedite prosecution have amended the claim to further clarify the claimed invention. Applicants note that the Patent Office required that:

"*Applicants must clarify what steps are required and whether they are performed sequentially or simultaneously*." Applicants note that a claim is not indefinite solely because it can be read to permit steps to be either simultaneous or sequential. The requirement under 35 USC 112 second paragraph is whether the claimed invention would be indefinite to those of skill in the art. In the cell culture methods of the invention, (i) adding cell culture medium to the cell culture, and (ii) circulating the cell culture through the pores of a filter module, are ongoing processes, as would be understood by those of skill in the art based on the recitation of "continuous perfusion culturing." As a result, (i) and (ii) may in some instances be sequential and in other instances be simultaneous. This in no way renders the claim indefinite to those of skill in the art.

**(g)** The Patent Office further "queried" on whether "alternating tangential flow" is a trade name of Refine Technology Company ("Refine"), or whether one of skill in the art would understand the full scope of the term. The Patent Office goes on to state "If the latter…applicants must support their assertion with at least one piece of patent or non-patent literature that employs the phrase in a manner other than in reference to Refine's apparatus." As an initial matter, Applicants note the following:

(1) The Patent Office has provided absolutely no evidence to show that "alternating tangential flow" is a trade name of Refine. Indeed, the reference cited by the Patent Office for this proposition does not make any indication whatsoever that Refine is asserting rights in "alternating tangential flow" (no use of the TM symbol or other indication of assertion of rights). Further, "alternating tangential flow" is simply a description of a cell culture process, and thus not protectable as a trade name for use in association with goods or services related to cell culture. A perusal of the Refine technology web site does not indicate any assertion of rights in the phrase "alternating tangential flow," instead indicating an assertion of rights in the acronym "ATF". Applicants have thus amended the specification to remove reference to the acronym "ATF" where it refers to alternating tangential flow.

(2) The Patent Office has provided absolutely no legal basis for its requirement for "at least one piece of patent or non-patent literature that employs the phrase in a manner other than in reference to Refine's apparatus." This is not surprising, since there is no legal basis for such a requirement by the Patent Office. In fact, Applicants' present specification is

one such piece of patent literature that employs the phrase in a manner other than in reference to Refine's apparatus. Such generic usage of the term not in relation to Refine's apparatus occur throughout, including but not limited to the title, the abstract, the claims as published, and paragraphs 2 and 10.

**(g)** Claims 2 and 3 were rejected under 35 USC §112, second paragraph, as being indefinite, based on various assertions. Applicants have canceled claims 2 and 3, thus obviating these rejections.

**(h)** Claim 4 was rejected under 35 USC §112, second paragraph, as being indefinite, based on the assertion that the recitation of "additional cell culture medium" being added to the cells was unclear, in that it might simply reference the cell culture medium of claim 1. Applicants traverse this rejection, as use of the term "additional" makes clear that the cell culture to be added is not the same as recited in claim 1. Nonetheless, solely in order to expedite prosecution, Applicants have amended the claim to even further clarify that the cell culture medium added is media added to compensate for biomass (e.g., cells in cell culture; see application as filed on page 5 line 14) removal. Thus, the claim would be clear to those of skill in the art, and Applicants respectfully request reconsideration and withdrawal of the rejection.

**(i)** Claim 5 was rejected under 35 USC §112, second paragraph, as being indefinite, based on the assertion that the recitation of "steady state" would be unclear to those of skill in the art. Applicants traverse this rejection, as the term "steady state" has a well-known meaning in the art. Nonetheless, solely in order to expedite prosecution, Applicants have amended the claim to further clarify. Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

**(j)** Claim 6 was rejected under 35 USC §112, second paragraph, as being indefinite, based on the assertion that its language was confusing. Applicants have amended the claim to obviate the rejection, and Applicants respectfully request reconsideration and withdrawal of the rejection.

**(k)** Claims 8 and 9 were rejected under 35 USC §112, second paragraph, as being indefinite, based on the assertion that it was unclear whether any additional method steps were required. Applicants note that claim 8 clearly requires that the "animal cells are cultured to a cell viability of at least 90%", while claim 9 clearly requires that "no more than 4% of the animal cells in the culture form aggregates of at least 5

cells during the continuous perfusion culturing". These are the additional limitations imposed by the claim, and there is nothing unclear about these claims; no other limitations are imposed by these claims, which would be understood by those of skill in the art upon review of the claims. That the claims recite end results does not make them indefinite, as these limitations would be clearly understood by those of skill in the art. Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

**(l)** Claim 15 was rejected under 35 USC §112, second paragraph, as being indefinite, based on the assertion that recitation of "further purified in downstream processing" is unclear, in that it could read on an intracellular process, such as 'ATP which is synthesized in mammalian cells and 'purified' as it moves out of the mitochondrion and into the cytoplasm." Applicants traverse, noting that "purifying" a biological product is a term well understood by those of skill in the art (and does not include ATP movement into a cell cytoplasm). Nonetheless, solely in order to expedite prosecution, Applicants have amended the claim to recite that the biological product is further purified from the cell culture, thus obviating the rejection. Applicants respectfully request reconsideration and withdrawal of the rejection.

**4. Claim rejections under 35 USC 103**

The Patent Office rejected claims 1-9 and 11-15 under 35 USC 103(a) as being obvious over Kyung et al. in view of Shevitz and Furey. Specifically, the Patent Office asserts that Kyung teaches continuous perfusion of mammalian cells in a bioreactor until the cells reach an approximate density of 100 x $10^6$ cells/ml, and also teaches optimizing the calcium concentration of the medium in order to reduce cell aggregation. Shevitz is cited for its teaching of alternating tangential flow, and is asserted to teach that its bioreactor eliminate large cell aggregates in cell culture. Furey is cited for its teaching of culturing cells that produce biological products in the Shevitz bioreactor. The Patent Office further asserts that the pending claims would be obvious to those of skill in the art by combining the teachings of Kyung, Shevitz, and Furey, and that "the skilled artisan would have had a reasonable expectation of confining cell aggregates to less than 5% of the total cell culture and few than 5 cells per aggregate because Kyung teaches that aggregate formation may be controlled by optimizing the contents of the culture medium and Shevitz' ATF bioreactor is specifically designed to eliminate cell aggregates and to inhibit their formation." Applicants traverse this rejection.

The PTO's *Examination Guidelines for Determining Obviousness* ("*Guidelines*") set forth seven exemplary rationales that may be used to establish a case of *prima facie* obviousness. MPEP 2141.III; MPEP 2143. The present obviousness rejection is based on one of those seven rationales: a teaching, suggestion, motivation or basis in the prior art for one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to predict the claimed invention.

The rationale to support a conclusion that the claim would have been obvious is that "a person of ordinary skill in the art would have been motivated to combine the prior art to achieve the claimed invention and that there would have been a reasonable expectation of success." *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1360, 80 USPQ2d 1641, 1645 (Fed. Cir. 2006).

The Patent Office's analysis is based on the assertion that the combination of Kyung and Shevitz make obvious to those of skill in the art on how to limit cell aggregation during alternating tangential flow cell culture, such that no more than 5% of the animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing. This assertion is completely incorrect.

**(A) Kyung's disclosure exemplifies the cell aggregation problems in the art that the present invention overcomes**: The Patent Office states that Kyung teaches optimizing the calcium concentration of the medium in order to reduce cell aggregates, pointing to page 188, first full paragraph. However, Kyung simply says that "293 cells form aggregates at high $Ca^{2+}$ concentration," without stating what constitutes a "high $Ca^{2+}$ concentration." Kyung then teaches use of medium comprising 100 um $Ca^{2+}$, and follows by stating "[h]owever, over a prolonged cultivation period, some large aggregates did form." Furthermore, Figure 4 shows confocal micrographs of such aggregates (120 hour culture sample and 300 hour culture sample), which show quite large clumps of cells that clearly contain many more than 5 cells, and which contain many dead cells. Thus, a close reading of Kyung makes clear that those of skill in the art would not have a reasonable expectation of success, based on the teaching of Kyung, in confining cell aggregates to less than 5% of the total cell culture and few than 5 cells per aggregate. In fact, Kyung exemplifies exactly the problem of cell aggregation that the present invention overcomes.

**(B) Shevitz provides no teaching at all about reducing cell aggregation:**

The Patent Office asserts that Shevitz' discloses that its bioreactor eliminate large **cell** aggregates in cell culture, pointing to teachings about two-way flow (pointing to column 3, lines 37-41; column 9 lines 64-66, and column 15 lines 7-12), and pointing to its teaching regarding filtering out the ones that do form (column 14 line 64 through column 15 line 7). However, the Patent Office has misinterpreted the teachings of Shevitz. In fact, a careful reading of Shevitz makes clear that Shevitz never mentions cell aggregates and is, in fact, referring to **particulate aggregates.** Taking each section recited by the Patent Office in turn, it is clear that none makes any mention of cell aggregates:

*Column 3, lines 37-41*

"*Recirculation in one direction through the hollow fiber cartridge typically results in clogging of the hollow fiber lumen by aggregates lodging at lumen inlet. Such aggregates may grow in size and as more hollow fibers are blocked, filtration capacity declines.*"

No mention is made of cell aggregates; in fact, this section says nothing about reducing any type of aggregate, but merely states that "aggregates" can lodge at the lumen and increase in size.

*Column 9 lines 64-66:*

"*Such a back-flush during each filtering cycle results in further clearing of particulates or gelatin build-up on the inner wall of the filter 18. The process can contribute significantly to the maintenance of filtration longevity.*"

This section cited by the Patent Office clearly is not referring to cell aggregates, but instead to particulates or gelatin build-up.

*Column 15 lines 7-12:*

"*In contrast, the longer the flow is maintained, continuously in one direction, the greater the probability that particles will become permanently lodged at the inlet end of the hollow fibers. The pulsating flow, back and forth between vessel and*

*pump inhibits both the attachment and growth of an obstruction at either end of the filter."* This section cited by the Patent Office clearly is not referring to cell aggregates, but instead to particles.

*Column 14 line 64 through column 15 line 7:*
*"The dynamics of the inventive system can extend the operating life of a perfusion run since pulsating flow between vessel 2 and chamber 30 greatly inhibit the attachment of aggregates to the hollow fiber lumen or to the filter membrane. For example, as culture medium flows from vessel 2 to pump 34, aggregates that are larger than the inside diameter of the hollow fibers will be retained by the hollow fiber array; i.e., the hollow fibers will act as a filter, however, by repeated and rapid reversal of flow direction, the deposited aggregates are quickly removed and swept back to the vessel."*

No mention is made of cell aggregates.

In order to understand what Shevitz means by aggregates, the following are the two other recitations of "aggregate" in the patent:

*Column 1 lines 54-57*

*"However, many of these filters have short operating lives, and when used to filter cell culture suspension or other biological fluids they tend to clog with* ***dead cells****, cell debris,* ***aggregates*** *or other constituents of the fluid."*

No mention is made of cell aggregates; in fact, the section appears to distinguish "aggregates" from dead cells/debris, indicating that "aggregates" refers to some type of non-cell aggregate, such as particulate debris (such as particulates or gelatin build-up referred to at column 9 lines 64-66, or "particles," as referred to at column 15 lines 7-12, both cited by the Patent Office).

*Column 2 lines 47-56*

*"Presently known perfusion methods which are used to separate a medium from cells frequently damage the cells. This damage may result from direct physical disruption by shearing forces of the system, depletion of nutrients in the medium, changes in physiological conditions of the culture, such as ionic strength, pH, etc., exposure to growth suppressing elements released by the cells. The resulting build up of* ***dead cells*** *and* ***aggregates*** *on screens or filters, resulting in clogging and failure of the perfusion device."*

Similarly, to Column 1 lines 54-57, no mention is made of cell aggregates; in fact, the section appears to distinguish "aggregates" from dead cells/debris, indicating that "aggregates" refers to some type of non-cell aggregate, such as particulate debris (such as particulates or gelatin build-up referred to at column 9 lines 64-66, or "particles

Thus, a close reading of Shevitz makes clear that those of skill in the art would not have found that the disclosed ATF bioreactor was specifically designed to eliminate cell aggregates and to inhibit their formation, as asserted by the Patent Office. Further, the combination of Kyung and Shevitz would not have provided those of skill in the art would not have a reasonable expectation of success in confining cell aggregates to less than 5% of the total cell culture and few than 5 cells per aggregate. Nothing in the Furey reference serves to overcome the deficiencies in Kyung and Shevitz, and thus the combination of all three references does not render the present invention obvious to those of skill in the art.

Further, nothing in the combination of cited references would provide a reasonable expectation of success to those of skill in the art that the continuous perfusion culturing could be continued until animal cells are present in the cell culture at a density of at least 80 x $10^6$ viable animal cells/ml, while at the same time limiting cell aggregation as recited in the claims. Neither Shevitz nor Furey provide any teaching or suggestion of cell densities of animal cells that can be achieved. Kyung teaches that at 400 hours of culture, a maximum of 9.3 X $10^7$ cells/ml were achieved (page 188, left column, near end of first paragraph bridging pages 187 and 188). However, Kyung also states that at 400 hours cell viability was 85% (page 188, left column, first full paragraph, third line from end of paragraph). 85% of 9.3 X $10^7$ cells/ml is 7.9 X $10^7$ cells/ml. Thus, one of skill in the art would not have had a reasonable expectation of success that the continuous perfusion culturing could be continued until animal cells are present in the cell culture at a density of at least 80 x $10^6$ viable animal cells/ml, particularly where no more than 5% of the animal cells in the culture comprise aggregates of at least 5 cells, as recited in the presently pending claims.

For all of the above reasons, the presently claimed invention would not be obvious to one of skill in the art based on the combination of references cited by the examiner. Furthermore, the present invention provides unexpected results that rebut any presumption of obviousness.

> "One way for a patent applicant to rebut a *prima facie* case of obviousness is to make a showing of 'unexpected results,' *i.e.*, to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. The basic principle behind this rule is straightforward—that which would have been surprising to a person of ordinary skill in a particular art would not have been obvious." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995).

Those of skill in the art would have found it surprising that the claimed methods could confine cell aggregates to less than 5% of the total cell culture and few than 5 cells per aggregate. As noted in the specification, this a surprising finding because low shear conditions, such as in continuous perfusion cell culturing typically do not lead to disaggregation of cells. Cell aggregation during perfusion cell culturing is disadvantageous, because process control is more difficult, due to, for example, the heterogeneity in metabolic profiles of cells within the cell aggregates. This is especially troublesome if cells form aggregates of 5 cells or more and when the aggregates comprise in total 5% or more of the total amount of cells.

Based on all of the above, it is clear that the claimed methods are not obvious over the combination of cited art. Thus, Applicants respectfully request reconsideration and withdrawal of the rejection.

If the Examiner has any concerns regarding this Response, he is encouraged to contact the undersigned attorney as indicated below at 312-913-2106.

Respectfully submitted,
**McDonnell Boehnen Hulbert & Berghoff LLP**

Date: /29 April 2011/

By: /David S. Harper/
David S. Harper, Ph.D.
Registration No: 42,636

Telephone 312-913-2106
Facsimile 312-913-0002